



Nuclear Extraction Kit

For Use with Transcription Factor Assays

User Manual
AY2002

Panomics, Inc.

Nuclear Extraction Kit User Manual

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About the User Manual

Who Should Read this Manual	Anyone that has purchased a Nuclear Extraction Kit from Panomics to prepare nuclear extracts for use in Panomics' Procarta TF, EMSA, TF ELISA or PD Array kits.
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What this Manual Covers	This manual provides the following: ◆ Kit contents ◆ Required materials and equipment ◆ Nuclear extraction procedure
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Safety Warnings and Precautions	CAUTION All chemicals should be considered potentially hazardous. We recommend that this product and its components be handled by those trained in laboratory techniques and be used according to the principles of good laboratory practice.
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	Note This product is intended for research use only.
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For More Information	For information about the Procarta products mentioned in this manual, visit our website at www.panomics.com .
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Nuclear Extraction Kit

About the Nuclear Extraction Kit	The Nuclear Extraction Kit contains reagents and procedures for the preparation of nuclear extracts for use in our Transcription Factor Assay kits. The Nuclear Extraction Kit contains sufficient reagents for the preparation of 80 nuclear extracts from cultured cells grown in 6-well culture plates or 20 nuclear extracts from cultured cells grown in 100-mm culture dishes.
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Kit Contents and Storage	The Nuclear Extraction Kit contains the following components. Nuclear Extraction Kit components:
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Component	Quantity	Storage
Buffer A	20.0 mL	-20 °C
Buffer B	12.0 mL	-20 °C
DTT, 100 mM	350 µL	-20 °C
Protease Inhibitor	350 µL	-20 °C
Phosphatase Inhibitor I	320 µL	-20 °C
Phosphatase Inhibitor II	320 µL	-20 °C

Required Materials and Equipment Not Provided

Materials and Equipment

Item	Source
1X PBS	Invitrogen (P/N 14190-144)
Rocking platform	VWR, Rocking Platform, Model 100 or equivalent
Centrifuge	Eppendorf (P/N 5804R)
Cell scraper (for adherent cell types)	Costar (P/N 3010) or equivalent
Protein determination kit	Bio-Rad DC Protein Assay Kit (P/N 500-0112) or equivalent
Microcentrifuge tubes	MLS
15 mL conical centrifuge tubes	MLS
Adjustable single and multi-channel precision pipettes	MLS

Cell Preparation

Growing Cells In all cases, cells are grown to ~90% confluence. The following table provides recommendations for the cell requirements for each culture vessel type. However, it is important to realize that cell types vary in size and actual numbers of cells/vessel may vary.

Use the table below as a guide.

Culture Vessel	Cell Number
100-mm culture dish	1 x 10 ⁷ cells/dish
6-well plate	1 x 10 ⁶ cells/well

Nuclear Extraction Procedure for Cultured Cells

Assay Guidelines **IMPORTANT** All components and PBS must be kept on ice at all times. Buffer A and B Working Reagents must be kept on ice and should be used within 2 hours of preparation.

Preparing Working Reagents

To prepare working reagents:

Step	Action						
1	Prepare 1 mL of Buffer A Working Reagent: - Combine: 1 mL Buffer A 10 μL DTT 10 μL Protease Inhibitor 10 μL Phosphatase Inhibitor I 10 μL Phosphatase Inhibitor II - Invert to mix. Scale preparation of Buffer A Working Reagent based on experimental requirements. Use the table below as a guide. <table> <tr> <th>Vessel</th><th>Quantity of Buffer A Working Reagent</th></tr> <tr> <td>100 mm culture dish</td><td>1 mL/dish</td></tr> <tr> <td>6-well plate</td><td>250 μL/well</td></tr> </table>	Vessel	Quantity of Buffer A Working Reagent	100 mm culture dish	1 mL/dish	6-well plate	250 μ L/well
Vessel	Quantity of Buffer A Working Reagent						
100 mm culture dish	1 mL/dish						
6-well plate	250 μ L/well						

To prepare working reagents: *(continued)*

Step	Action						
2	Prepare 1 mL of Buffer B Working Reagent: - Combine: 1 mL Buffer B 10 μL DTT 10 μL Protease Inhibitor Cocktail 10 μL Phosphatase Inhibitor I 10 μL Phosphatase Inhibitor II - Invert to mix. Scale preparation of Buffer B Working Reagent based on experimental requirements. Use the table below as a guide. <table> <tr> <th>Vessel</th><th>Quantity of Buffer B Working Reagent</th></tr> <tr> <td>100 mm culture dish</td><td>150 μL/dish</td></tr> <tr> <td>6-well plate</td><td>150 μL/well</td></tr> </table>	Vessel	Quantity of Buffer B Working Reagent	100 mm culture dish	150 μ L/dish	6-well plate	150 μ L/well
Vessel	Quantity of Buffer B Working Reagent						
100 mm culture dish	150 μ L/dish						
6-well plate	150 μ L/well						

Preparing Nuclear Extracts From Adherent Cells

To prepare nuclear extracts:

Step	Action						
1	Remove the culture media from all wells and wash cells twice with an appropriate volume of cold 1X PBS. <table> <tr> <th>Vessel</th><th>Quantity of PBS</th></tr> <tr> <td>100 mm culture dish</td><td>10 mL/dish</td></tr> <tr> <td>6-well plate</td><td>1 mL/well</td></tr> </table>	Vessel	Quantity of PBS	100 mm culture dish	10 mL/dish	6-well plate	1 mL/well
Vessel	Quantity of PBS						
100 mm culture dish	10 mL/dish						
6-well plate	1 mL/well						
2	Following the second wash, make sure the PBS is completely removed.						
3	Add the appropriate volume of Buffer A Working Reagent to the wells. <table> <tr> <th>Vessel</th><th>Quantity of Buffer A Working Reagent</th></tr> <tr> <td>100 mm culture dish</td><td>1 mL/dish</td></tr> <tr> <td>6-well plate</td><td>250 μL/well</td></tr> </table>	Vessel	Quantity of Buffer A Working Reagent	100 mm culture dish	1 mL/dish	6-well plate	250 μ L/well
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100 mm culture dish	1 mL/dish						
6-well plate	250 μ L/well						
4	Transfer culture vessel(s) to an ice bucket and transfer ice bucket to a rocking platform at 200 rpm for 10 minutes.						
5	Release the cells from the bottom of culture vessel: - Using a sterile cell scraper, remove the cells - Pipet up and down several times to disrupt the cell clumps. (Avoid creating bubbles). - Rinse the bottom of the culture vessel with the cell suspension to maximize the cell yield.						
6	Transfer each sample to a 1.5 mL microcentrifuge tube and centrifuge at 14,000 x g for 3 minutes at 4 °C.						
7	Remove and discard the supernatant(s) and keep the pellet(s) on ice.						

To prepare nuclear extracts: (continued)

Step	Action						
8	Add 150 μ L of Buffer B Working Reagent to each pellet and vortex at highest setting for 10 seconds. The pellet will detach from the microcentrifuge tube wall and may not disperse into a homogenous solution. This is normal. Do not attempt to disperse the pellet.						
9	Position the microcentrifuge tubes horizontally in an ice bucket and transfer ice bucket to a rocking platform at 200 rpm for 2 hours.						
10	Centrifuge samples at 14,000 x g for 5 minutes at 4 °C.						
11	Transfer supernatant(s) to a new microcentrifuge tube. This is your nuclear extract.						
12	Measure the protein concentration of each sample using a protein quantitation assay. Then prepare 5 μ L aliquots for each of the samples. Store samples at –80 °C or use immediately in Procarta TF Assay Kit.						
	<table border="1"> <thead> <tr> <th>Vessel</th><th>Typical Protein Yields</th></tr> </thead> <tbody> <tr> <td>100 mm culture dish</td><td>150–300 μg/dish</td></tr> <tr> <td>6-well plate</td><td>50–100 μg/well</td></tr> </tbody> </table>	Vessel	Typical Protein Yields	100 mm culture dish	150–300 μ g/dish	6-well plate	50–100 μ g/well
Vessel	Typical Protein Yields						
100 mm culture dish	150–300 μ g/dish						
6-well plate	50–100 μ g/well						

Preparing Nuclear Extracts From Suspension Cells

To prepare nuclear extract for suspension cells:

Step	Action						
1	Transfer cells to a 1.5 mL or 15 mL centrifuge tube as appropriate and centrifuge at 500 x g for 5 minutes.						
2	Remove the culture media and wash cells by resuspending in 1 mL of cold 1X PBS followed by centrifugation at 500 x g for 5 minutes. Repeat wash step. Following the second wash step, ensure that the 1X PBS solution is completely removed from the cells. Note Before the second centrifugation, if necessary, transfer contents from the 15 mL centrifuge tube to a 1.5 mL microcentrifuge tube.						
3	Immediately add the appropriate volume of Buffer A Working Reagent to cell pellets. Mix by pipetting up and down several times.						
	<table border="1"> <thead> <tr> <th>Vessel</th><th>Buffer A Working Reagent</th></tr> </thead> <tbody> <tr> <td>100 mm culture dish</td><td>1 mL/dish</td></tr> <tr> <td>6-well plate</td><td>250 μL/well</td></tr> </tbody> </table>	Vessel	Buffer A Working Reagent	100 mm culture dish	1 mL/dish	6-well plate	250 μ L/well
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100 mm culture dish	1 mL/dish						
6-well plate	250 μ L/well						
4	Transfer tube(s) to an ice bucket and transfer ice bucket to a rocking platform at 200 rpm for 10 minutes.						
5	Centrifuge samples at 14,000 x g for 3 minutes at 4 °C.						
6	Discard the supernatant(s) and keep the pellet(s) on ice.						
7	Add 150 μ L of Buffer B Working Reagent to each pellet and vortex at highest setting for 10 seconds. The pellet will detach from the microcentrifuge tube wall and may not disperse into a homogenous solution. This is normal. Do not attempt to disperse the pellet.						
8	Position the microcentrifuge tubes horizontally in an ice bucket and transfer ice bucket to a rocking platform at 200 rpm for 2 hours.						
9	Centrifuge samples at 14,000 x g for 5 minutes at 4 °C.						

To prepare nuclear extract for suspension cells: *(continued)*

Step	Action						
10	Transfer supernatant(s) to a new microcentrifuge tube. This is your nuclear extract.						
11	Measure the protein concentration of each sample using a protein quantitation assay (sold separately). Then prepare 5 µL aliquots for each of the samples. Store samples at –80 °C or use immediately in Procarta TF Assay Kit. <table border="1"> <thead> <tr> <th>Vessel</th><th>Typical Protein Yields</th></tr> </thead> <tbody> <tr> <td>100 mm culture dish</td><td>150–300 µg/dish</td></tr> <tr> <td>6-well plate</td><td>50–100 µg/well</td></tr> </tbody> </table>	Vessel	Typical Protein Yields	100 mm culture dish	150–300 µg/dish	6-well plate	50–100 µg/well
Vessel	Typical Protein Yields						
100 mm culture dish	150–300 µg/dish						
6-well plate	50–100 µg/well						

Nuclear Extraction Procedure for Whole Tissue

Assay Guidelines **IMPORTANT** All components and 1X PBS must be kept on ice at all times. Buffer A and B Working Reagents must be kept on ice and should be used within 2 hours of preparation.

About Whole Tissue Extraction Each gram of dry tissue should yield approximately 2×10^7 cells for the isolation of nuclei.

Each kit provides enough solution to prepare 5 grams of tissue.

Preparing Working Reagents To prepare Working Reagents:

Step	Action
1	Prepare 1X Buffer A, Buffer B, and PBS using nuclease-free, sterile water. Note 1X preparations are good for 6 months at 4 °C.
2	Prepare Buffer A Working Reagent in a clean, sterile tube. - Combine the following for each 0.5 grams of tissue: 2.5 mL 1X Buffer A 25 µL 100 mM DTT 25 µL Protease Inhibitor 25 µL Phosphatase Inhibitor I 25 µL Phosphatase Inhibitor II - Invert to mix. - Keep on ice.

To prepare Working Reagents: *(continued)*

Step	Action
3	Prepare Buffer B Working Reagent in a clean, sterile 1.5-mL tube. - Combine the following for each pellet: – 145.5 μL 1X Buffer B – 1.5 μL Protease Inhibitor – 1.5 μL Phosphatase Inhibitor I – 1.5 μL Phosphatase Inhibitor II - Invert to mix. - Keep on ice.

Preparing Nuclear Extract From Whole Tissue

To prepare nuclear extracts from whole tissue:

Step	Action
1	Prepare initial homogenate: - Weigh at least 0.5 grams of tissue and dice into very small pieces using a clean razor blade. - Place pieces into a pre-chilled, clean Dounce homogenizer. - Homogenize the sample with 15–20 strokes and keep on ice.
2	On ice, add 1.5 mL of Buffer A Working Reagent per each 0.5 gram of tissue and homogenize with another 15–20 strokes.
3	Incubate on ice for 15 minutes.
4	Centrifuge at 850 x g for 10 minutes at 4 °C. Discard supernatant. Note At this point, most cells have not been lysed.
5	On ice, add a second 1.5 mL of Buffer A Working Reagent per each 0.5 gram of tissue and homogenize with another 15–20 strokes.
6	Incubate on ice for 15 minutes.
7	Transfer the homogenate to microcentrifuge tube(s) and centrifuge at 14,000 x g for 3 minutes at 4 °C.
8	Remove and save (or discard) the supernatant (cytosolic fraction) and place pellet(s) on ice. If desired, store the pellet at –80 °C and, complete the protocol the following day.
9	Add 150 μ L of Buffer B Working Reagent to each pellet and vortex at the highest setting for 10 seconds. The pellet will detach from the microcentrifuge tube wall and may not disperse into a homogenous solution. This is normal. Do not attempt to disperse the pellet.
10	Position the microcentrifuge tubes horizontally in an ice bucket and transfer the ice bucket to a rocking platform at 150 rpm for 2 hours.
11	Centrifuge samples at 14,000 x g for 5 minutes at 4 °C.
12	Measure the protein concentration of each sample using a protein quantitation assay. Then prepare 5 μ L aliquots for each of the samples. Store samples at –80 °C or use immediately.

Contacting Panomics

Technical Help	For technical questions, contact our technical support group by telephone at 1-877-726-6642 option 3 or by email at techsupport@panomics.com (US and Canada) techsupport_europe@panomics.com (Europe), or visit our website www.panomics.com for an updated list of FAQs and product support literature.
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For Additional Services	For information about Panomics products or for ordering information, contact your Regional Sales Manager, or visit our website at www.panomics.com .
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